

## PHOTOPERIOD CONTROL OF DIAPAUSE IN *DAPHNIA*. II. INDUCTION OF WINTER DIAPAUSE IN THE ARCTIC

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The sexual polymorphism in *Daphnia* which results in an embryonic diapause is facultative, and its onset may be under the control of photoperiod and a density proportional stimulus (Stross and Hill, 1965, 1968). In the previous study a population of *D. pulex* which overwinters in an embryonic diapause at temperate latitude (45° N) responded to culture density when the photoperiod was 13 hours of light per day. At an appropriate density a photoperiod response curve, typical of other arthropods, was generated. The critical photoperiod was also temperature compensated in the range tested, 12° and 19° C. This report describes an extension of that study to arctic latitudes where an effort was made to determine if the reproductive cycle of arctic *Daphnia* may be under photoperiod control.

Facultative diapause is not readily apparent in arctic populations of arthropods from descriptions of life history. The brief summer season may allow only a single generation of insects to develop. At Barrow, Alaska (71°) a fresh-water calanoid copepod (Comita, 1956) and the Cladoceran, *Daphnia middendorffiana* Fischer (Edmondson, 1955) are reported to develop essentially only one generation each year. Univoltinism is not necessarily obligatory in arthropods at high latitudes as has been demonstrated (Danilevskii, 1965; Morris, 1967). Indeed the reproductive cycle of *D. middendorffiana* in a lake includes one or two broods of non-diapausing embryos before the population shifts. Populations of the same species at Cape Thompson, Alaska (68° N), where the ice-free season is longer are reported to shift at approximately the same time in the late autumn of the arctic (Hilliard and Tash, 1966).

The species *D. middendorffiana* is apparently widely distributed in the myriad small pools characteristic of the frost-patterned earth of the arctic. Brooks (1957) describes it as phylogenetically distinct from *D. pulex* which he excludes from the arctic. The only other species reported by him to occur in arctic Alaska near Barrow is *D. longiremis* a much smaller species which apparently overwinters in a non-embryonic stage. The second species is characteristic of lakes, and, with the exception of Imikpuk Lake which contains *D. middendorffiana* and fairy shrimp but no fish or other dangerous predators, it may be found in fish-containing lakes such as Ikroavik (Wohlschlag, 1957) and Sungoroak near Barrow.

### METHODS AND MATERIALS

Daylength, culture density and temperature were tested as potential stimuli controlling the reproductive shift of *Daphnia middendorffiana* Fischer. The laboratory experiments were complemented by simultaneous observation of the reproductive cycle in the arctic pool from which the experimental stock was removed.

The study began on June 15 at Barrow when overwintering embryos, still in their egg pods (ephippia) were transferred to the laboratory. The embryos hatching in continuous light were used in the first experiments and to provide an uncloned stock for measurement of response in the first and second generations.

Culture techniques were as described previously (Stross and Hill, 1968). They consist of rearing young, 0 to 2 days of age for a period of usually 30 days at controlled densities. At two-day intervals the test animals are transferred to fresh suspensions of food, and released broods are censused. Water from the source pool served as culture medium after it had been filtered through a plankton net (mesh opening, 50 microns). A synthetic medium, previously described was used as a control. The food medium consisted of a mixture of axenically grown cells of *Chlamydomonas reinhardtii* (Indiana U. strain #90) and a nearly unialgal suspension of *Kirchneriella (subsolaria?)* which was grown in a greenhouse aquarium under natural daylight; the mixture was used with some indication that *Chlamydomonas* alone might be unsatisfactory. Initial cell densities ranged from 0.9 to  $1.2 \times 10^6$  cells/ml in the cultures during the course of the investigation.

The *Daphnia* cultures were housed in water baths at  $12.5$  or  $20.0 \pm 1.0^\circ$  C. Illumination, controlled by electric timers, was supplied as fluorescent (cool white) light at intensities of 125 and 28 ft-c at the level of the cultures.

Field observations consisted of weekly collections of duplicate samples from the source pool (Near Ditch) and in semi-weekly census of small (400 ml) transparent enclosures suspended in the source pool. Dimensions of the pool in June were approximately 3 meters  $\times$   $1\frac{1}{2}$  meters  $\times$   $\frac{1}{2}$  meter deep (maximum). The volume later shrank as a result of evaporation and withdrawal of medium for the laboratory experiments. Temperatures of the pool were recorded with a Taylor max-min thermometer and Foxboro recording thermometer. Details of water chemistry, algal populations, etc. have been described for the arctic pools near Barrow (Kalff, 1967).

The reproductive cycle of *Daphnia* was examined in three other small pools near the Arctic Research Laboratory and in Imikpuk, Ikroavik and Sungroak lakes near Barrow, Alaska. A single observation was made on the status of the reproductive cycle in one of the lakes at Cape Thompson ( $68^\circ$  N) previously studied by Hilliard and Tash (1966).

## RESULTS

The reproductive shift which leads to the diapausing embryo may be determined by daylength. Photoperiod control is expressed more clearly by the generations born to the overwintering generation. All broods are (ephippial) diapausing at photoperiods of 20 hours (L:D 20:4) or shorter including only 15 minutes of light per day (Fig. 1). The incidence or fraction of diapausing broods at daylengths longer than 20 hours is influenced by density of culture. At the minimum density of one per container (20 ml), no broods (5 replicates) are diapausing in constant light. Densities of 3 or 5/20 ml result in 20 or 50 per cent, respectively, diapausing broods in constant light. A critical photoperiod (ratio of 1:1 diapausing to non-diapausing broods for the test period) is approximately L:D 22:2 as shown by the response at densities of 1 and 3/20 ml. The densest cultures (5/20 ml) resulted in an apparent shift of the critical photoperiod to a longer daylength (Fig. 1).

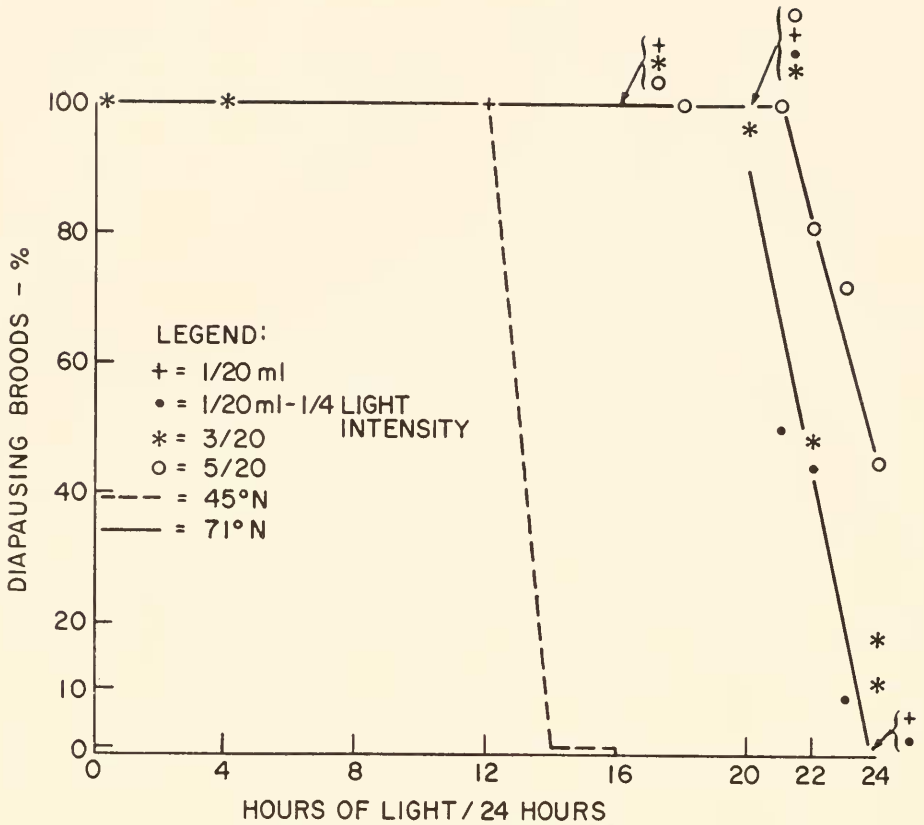


FIGURE 1. Reproductive shift of *Daphnia middendorffiana* in response to daylength and culture density. First and second-generation young born in constant light and transferred to photoperiod indicated. Photoperiod response of winter diapausing population of *D. pulex* at 45° N is shown for comparison. All experiments at 12° C.

Starvation may be discounted as a stimulus associated with culture density. All densities received the same bi-daily ration of food, and this is reflected in the number of young in broods of non-diapausing embryos. At 12° mean brood size for the test interval, which includes the first four or five broods of the adult reproductive period was 13.3 young brood at a density of 1/20 ml and 5.5 and 3.2 young at densities of 3 and 5/20 ml, respectively, in constant light.

#### *Overwintering generation*

Daylength and density may also control the reproductive shift in the overwintering generation. These stimuli are effective, however, only after the adults have produced one or two broods of non-diapausing embryos (or young) and under conditions which are inductive for first and second generation adults. Young which had spent most of their prenatal development in constant light, either in the field or in the laboratory were subjected to four photoperiods at densities of

3, 5 and 10/20 ml. The food ration was doubled for the densest cultures. The incidence of diapause increased with decreasing daylength and with increasing density. Both variables were significant ( $P = 0.01$ ) in a two-way analysis of variance (5 replicates) at both 12 and 20° C. Although significant, the variables clearly fail to generate the elegant all-or-none response provided when generations of the year were tested.

The number of non-diapause broods produced by the overwintering generation before the reproductive shift is variable and may be determined by photoperiod and culture density. At 12° and constant light the first two broods produced by each female at minimum density (3/20 ml) were virtually all (26 of 27) non-diapausing (Fig. 2). Denser cultures produced fewer non-diapausing broods such that at a density of 10 adults/20 ml most of the females released only one brood of young before switching. At inductive photoperiods the first brood was non-diapausing in all but the densest cultures. Broods subsequent to the first were nearly all diapausing at inductively short photoperiods. In constant light, the only non-inductive photoperiod tested, some of the third or later broods were non-diapausing even in the densest cultures. The response to photoperiod and density was similar at 19° that is, the temperature effect in a three-way ANOV was not significant.

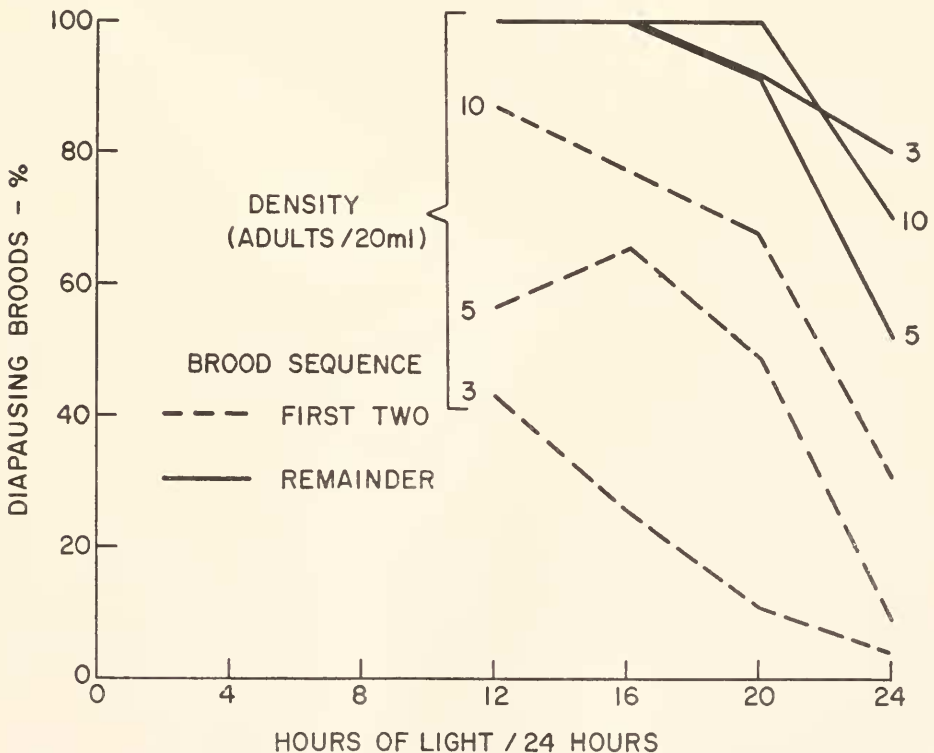


FIGURE 2. Reproductive shift of overwintering generation of *D. middendorffiana* in response to photoperiod and density at a temperature of 12° C.

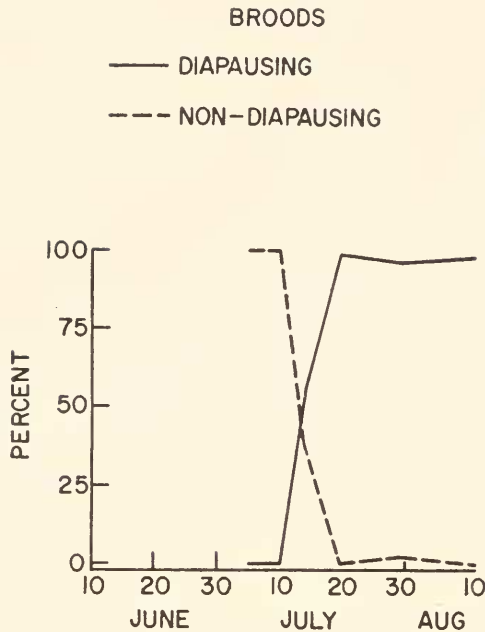


FIGURE 3. Reproductive shift of *D. middendorffiana* in the arctic pool supplying laboratory stock, as determined by inspection of brood contents of the adults. The sun is continuously above the horizon until August 3.

### *Males*

Males are reported to be non-functional in *D. middendorffiana* (Edmondson, 1955; Brooks, 1957), as determined from examination of the testes. These conclusions are supported in this study by the successful rearing of the diapaused embryos developed by females isolated since birth. Although rare in nature, males are produced. In the source pool, the mid-August collection contained 4.8 per cent males. Hosseinie (1966) reports having obtained them regularly in cultures. In this study a single male was discovered to have been produced in culture, of some 1500 first and second generation individuals used in the experiments.

### *Reproductive cycle in the field*

In the laboratory the reproductive switch may be judged facultative and under the control of photoperiod. The critical photoperiod of L:D 22:2 would permit the reproductive shift to begin on or about September 1, depending on the requisite number of inductive light-dark cycles required by the adults. A shift at that time is consistent with the major shift reported by Edmondson (1955) for Imikpuk Lake at Barrow and by Hilliard and Tash (1966) for small lakes at Cape Thompson, Alaska (68° N).

In the pool environment the reproductive shift is in mid-July, two weeks before the first sunset of the summer and six weeks earlier than a direct extension of laboratory results would permit. The mid-July shift was recorded for the source

pool (Fig. 3) and in two other nearby pools. A fourth pool deepened by previous passage of tracked vehicles shifted in late July. In the source pool the population apparently produced only one brood of non-diapausing embryos before shifting or 2.65 young per adult, as measured in the exclusion containers. They were released during the interval of July 10 and 17. During the following month, or from July 17 to August 15, each adult produced 2.3 diapausing embryos (1.4 egg pods) most of them during the first two weeks after the shift.

On a seasonal basis the synchrony in population growth and development is highly compressed. In 1967 overwintering embryos hatched on June 19 and 20 one week after the pool was free of ice and on the same days that embryos transferred to the laboratory hatched when held at 11.5°. Two weeks later oviposition into the brood pouch began; 7 per cent of the population was gravid on July 5, and 48 per cent were carrying embryos two days later. Essentially all of the young were born between July 10 and 17. The reproductive shift was equally compressed. On July 10 all gravid females were carrying non-diapausing embryos (Fig. 3). Four days later 55 per cent of the gravid females were developing broods of diapausing embryos; 80 per cent of the adults were gravid at this time. On July 17, egg pods (ephippia) containing the diapausing embryos were being released and 95 per cent of the developing broods were diapausing. The density of *Daphnia* on July 10 was estimated at approximately 60/liter.

Development is more rapid in the pools, than in Imikpuk Lake (Edmondson, 1955). Temperatures in the pools averaged 10° or 11° from mid-June until the end of July. The daily oscillation ranged from 4° to 7° at approximately 0300 to 12° to 15° C at 1500 hours; the oscillation may be damped near either extreme by the time of day when clouds or fog blanket the earth. In Imikpuk the temperature lags such that a maximum temperature of 10° is not attained until late July following the disappearance of ice on July 19 (Edmondson, 1955).

The timing of the reproductive shift in Imikpuk is confirmed in this study. Gravid female *Daphnia* collected on August 15 contained only broods of non-diapausing embryos. On August 29 the population was in transition since 30 per cent of the gravid females were carrying broods of diapausing embryos. Gravid females in the population in a lake at Cape Thompson contained 38 per cent diapausing broods when sampled three days earlier.

#### *Reversal of reproductive shift*

Reproduction may revert to non-diapause when the females are placed in the appropriate environment. In the first experiment females from overwintering embryos were diluted from a density of 10/20 ml to a density of 3/20 ml after they had released the first brood. In constant light the incidence of diapausing broods was reduced to 43.5 per cent as compared with 92.0 per cent in undiluted controls. There was no reversal following dilution at L:D 20:4. In the second experiment adults were transferred from the source pool on August 1 where they had been under continuous light. Cultured at a density of 1/20 ml in constant light, the females reverted to an extent predicted by the photoperiod response curve (Fig. 1). In constant light 3 of 4 reverted to the production of non-diapausing embryos. At L:D 20:4 and 21:3 none reversed, while at 22:2, 2 of 4 reverted. The response was the same at both light intensities.

The response of the field-collected animals differed in the time required for reversal to occur. The laboratory crowded animals (Expt. 1) reverted quickly and there was no difference in incidence of diapausing broods in the first and second 15-day intervals following dilution. Animals reared in the pools required 22 or 26 days to release the first non-diapausing broods. Both experiments were carried out at 12° C.

#### *Synchrony in laboratory and field*

Several lines of evidence indicate a moderate degree of synchrony in field populations in the presence of continuous light. Embryos transferred to the laboratory at constant temperatures hatched synchronously. At 12° 95 per cent (271) of the total hatched in 24 hours. At 20° the same percentage (231 young) hatched within 12 hours. Molting and release of brood-pouch contents among five replicates of single individuals, brought in as young from the source pool, remained in step, at least within the two-day interval between censuses (Fig. 4).

#### *Instar duration and reproductive polymorphism*

The synchrony of molting and release of broods of young or diapaused embryos (Fig. 4) permits an analysis of instar duration. The interval between successive broods of young was 4.7 days at 13° C. The interval between the release of an egg pod (ephippium) was 6.0 days at 13° C. However, the actual duration of a maternal instar which produces a brood of diapausing embryos is only one-half of the 6 days or 3 days. A barren instar intervenes between instars producing diapausing broods (not shown in Fig. 4) such that the instar is roughly two-thirds the length of an instar producing a brood of young. The intervening barren instar, apparent from the shed exuvium was first pointed out by Leary (1967) who reported barren instars for a population of *D. pulex* (Paul Lake, Mich.). Relative duration of instars was reported to be the same at 11° C. At 20° C diapausing instars were only 76 per cent as long. The longer duration required for release of a brood of diapausing embryos and the lack of fractional days in the average interval between release suggest the ovarian cycle is more rigidly entrained to a phase of the light-dark cycle.

### DISCUSSION AND CONCLUSIONS

The reproductive polymorphism in an arctic (71° N) population of *Daphnia middendorffina* is facultative, and the expression which leads to the development of diapausing embryos is under the control of environment. In the laboratory under constant temperature and standardized densities, photoperiod controls the reproductive shift. A critical photoperiod of L:D 22:2 is the longest known and nine hours longer than the critical photoperiod of 13:11 for a population of *Daphnia pulex* at 45° N (Stross and Hill, 1968). In responding the arctic population provides additional evidence for photoperiod involvement in the reproductive shift. Since the most dilute culture of one individual in 20 ml gave a complete response, there is no proof that a crowding stimulus is required in conjunction with a permissively short photoperiod. Nevertheless, the much longer critical photoperiod is evidence for latitudinal adjustment shown for other arthropods at mid and high

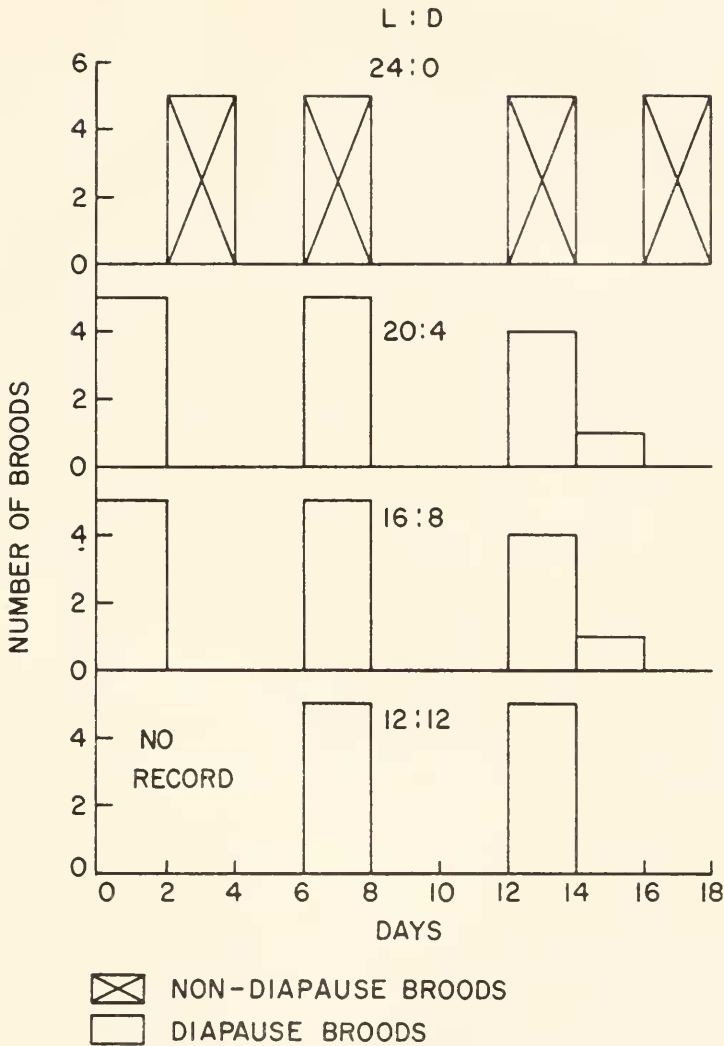


FIGURE 4. Synchrony of brood release in five replicates of single individuals per container at photoperiods indicated. Cultures examined at bi-daily intervals. Results plotted as the number of broods released during a two-day interval between inspection. *Daphnia middendorffiana* collected as first generation young from source pool and cultured at 12° C.

latitudes (Danilevskii, 1965), but not at low latitudes (Ankersmit and Adkisson, 1967) and required by Hutchinson (1967) for a general acceptance of photoperiod control of the reproductive polymorphism in Cladocera.

In the field, populations shift in the source pool near Barrow well in advance of prediction, and it might be argued that crowding overrides photoperiod control, as it may in the laboratory. Several facts argue against this, however. The shift may be avoided in the laboratory cultures containing water from the same pool. The density of *Daphnia* in the pool was only slightly greater than 1 adult/20



ml which gave no diapausing broods in constant light and much less than 3/20 which gave only partial overriding in constant light. Indeed a density of 10/20 ml permitted some non-diapausing broods. Yet in the field the shift was virtually complete.

Starvation is another possibility since a food supplement was added to the cultures. At the time of the shift in the pool, 80 per cent of the adults were gravid and that level of nutrition argues strongly against starvation.

The most likely candidate interacting with photoperiod to cause a reproductive shift is temperature. Either the low temperature or the large thermal oscillation may interfere with a photoperiod effected process as shown for insects (Pittendrigh, 1954; Saunders, 1967, 1968). Admittedly speculative, the last possibility is offered to counteract the inference that density is the most likely possibility by extension of the laboratory result. Thermal interaction with a long-day stimulus has many attractive possibilities. It could permit selection for a long critical photoperiod in arctic pools which, rather than the lakes, are probably the ancestral and certainly are the present principal location of the species. Thermal interference could explain the dramatic shift from all non-diapausing to virtually all diapausing broods in the pools. The slow reversal of *Daphnia* reared in the field suggests actual reversal of short-day induction and contrasts with the rapidity with which laboratory cultures responded when the suppressive effect of crowding was withdrawn in a long-day environment. In other words the populations in the pools may behave as if they spend the entire active phase of the life cycle in a short-day environment despite the continuous light. The resistance of the overwintering generation of *Daphnia* to short-day photoperiods is analogous to the response of females from overwintering embryos (fundatrix) in aphids (Lees, 1966) another seasonally polymorphic group. In *Daphnia* the resistance is restricted apparently to permitting birth of only one brood of young each year. The significance of population expansion each year and proof for the temperature postulate require further analysis.

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#### SUMMARY

1. Control of the sexual polymorphism leading to an embryonic diapause was studied in an arctic (71° N) population of *Daphnia middendorffiana*. The reproductive cycle is similar to a previously analyzed population of *D. pulex* although males are non-functional in the polymorphism.

2. In constant temperatures and standardized culture density the reproductive shift is controlled by daylength with a critical photoperiod of L:D 22:2 at 12° C. Control may be partially overridden in long days (constant light) by density of culture. The overwintering generation shows resistance to both photoperiodic

induction and density suppression, and one or two non-diapause broods are released before reproduction shifts.

3. The reproductive shift in the source pool at Barrow, Alaska occurs in mid-July when the sun is continuously above the horizon and after the overwintering generation has released one brood of non-diapausing embryos. Completeness of the shift and the relatively low density of the population in the pool argues against a density override of photoperiod control. Another component of the environment which may create effectively short daylengths in the presence of continuous light is postulated. The large oscillation in temperature or low minimum which occur daily could be involved. The reproductive shift in a lake at Barrow and at Cape Thompson (68° N) is consistent with photoperiod control.

4. Results of this study support the previous hypothesis in part, namely, that the sexual polymorphism in short-day induced populations of *Daphnia* may be under the control of photoperiod. Also shown is the latitudinal adjustment necessary for functioning of photoperiod at mid and high latitudes.

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